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(54) Title: HUMAN C5A-LIKE RECEPTOR

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      9      18      27      36      45      54
AGC TCA TGC TTC TCT GAA GAC TTG CAG CAA GGC TTG CTG AGG CTC ACA GAA GAT

      63      72      81      90      99      108
AGC CCC AGT GTT TTG GAG TGG TTT TGA ATG TGA TTC TGA GAT CAG ACT GAC TGA

      117     126     135     144     153     162
GCT GGA ATC CTG GCT TTA TAT CTT ACC AGC TAC WCA ACC TTG GAG TCT TAG AAA

      171     180     189     198     207     216
TTT TTT CTT TTC ART AAG CAG TCA TCC TTA CTT TCC CTC AAG ATG ACA AAC AGT
                        M   T   N   S

      225     234     243     252     261     270
TCG TTC TTC TGC CCA GTT TAT AAA GAT CTG GAG CCA TTC ACG TAT TTT TAT
S   F   F   C   P   V   Y   K   D   L   E   P   F   T   Y   F   F   Y

      279     288     297     306     315     324
TTA GTT TTC CTT GTT GGA ATT ATT GGA AGT TGT TTT GCA ACC TGG GCT TTT ATA
L   V   F   L   V   G   I   I   G   S   C   F   A   T   W   A   F   I

      333     342     351     360     369     378
CAG AAG AAT ACG AAT CAC AGG TGT GTG AGC ATC TAC TTA ATT AAT TTG CTT ACA
Q   K   N   T   N   H   R   C   V   S   I   Y   L   I   N   L   L   T
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(57) Abstract

The present invention provides a human C5a-like receptor (HCOR) and polynucleotides which identify and encode HCOR. The invention also provides genetically engineered expression vectors and host cells comprising the nucleic acid sequences encoding HCOR and a method for producing HCOR. The invention also provides agonists, antibodies, or antagonists specifically binding HCOR, and their use, in the prevention and treatment of diseases associated with expression of HCOR. Additionally, the invention provides for the use of antisense molecules to polynucleotides encoding HCOR for the treatment of diseases associated with the expression of HCOR. The invention also provides diagnostic assays which utilize the polynucleotide, or fragments or the complement thereof, and antibodies specifically binding HCOR.

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## HUMAN C5A-LIKE RECEPTOR

## TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of a novel human C5a-like receptor and to the use of these sequences in the diagnosis, prevention, and treatment of diseases and conditions associated with inflammation.

## BACKGROUND ART

Inflammation is a rapid, nonspecific, and complex response to cellular injury. The inflammatory process may be triggered by damage to cells induced by a variety of factors. The initial cellular and biochemical responses originate from components found in mast cell granules and include vasodilation and an increase in vascular permeability. These local alterations allow cells, platelets, and plasma proteins to migrate from the blood vessels into the injured tissues. Acting through receptor-mediated processes, the cells and plasma proteins both stimulate and control the subsequent inflammatory reactions and interact with components of the immune system.

The stimulated cells release a variety of factors including vasoactive amines, which maintain vascular permeability, and chemotaxic factors, which attract various types of leukocytes. These cells produce factors that bind to cellular receptors and mobilize additional components of the inflammatory and the immune systems. The plasma proteins which infiltrate the tissue are components of the kinin, clotting, and complement systems. These proteins occur as inactive forms and can be activated by antigen-antibody complexes. The cells and plasma protein systems, along with the factors that they produce, induce the physiological responses necessary to kill microorganisms, remove damaged tissues, and prepare the region for tissue repair or regeneration.

The activated components of the complement system are participants in most of the inflammatory response processes. In particular, components C1 to C5 act as chemotaxins, C6 through C9 as opsinins, and C3 to C5 as anaphylatoxins which induce mast cell degranulation. These molecules mediate biological responses via the activation of cell surface receptors that are coupled to phospholipase C through G proteins. The most potent of these inflammatory mediators, C5a, binds to the C5a receptor to elicit chemotaxis of neutrophils, eosinophils, basophils, macrophages, and monocytes. In addition, C5a induces degranulation, production of superoxides, and vasculature permeability; its activities are potentiated by prostaglandins and circulating leukocytes. In contrast, other inflammatory mediators such as Rantes, IL-8, histamines, and bradykinin may have vascular but not chemotaxic effects, and those that do elicit

chemotaxis attract fewer cell types (Gerard, N. and Gerard, C. (1991) Nature 349:614-617; Boulay, F. et al. (1991) Biochemistry 30:2993-2999).

The human C5a receptor has been cloned and has been characterized as a member of the G protein-coupled seven transmembrane family. Stimulation of this receptor produces the pleiotropic effects which are necessary for inflammatory response and tissue repair but also causes tissue damage, allergic responses, and inappropriate immunologically-mediated responses (Gerard N. and Gerard, C. supra; Gerard, C. and Gerard, N. (1994) Annu Rev Immunol 12:755-808).

Complement-mediated tissue damage is associated with brain demyelination and neurodegeneration, allergic reactions, asthma and adult respiratory distress syndrome, autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, glomerulonephritis, and Crohn's disease, post ischemic myocardial inflammation and necrosis, skin diseases, and septic shock. In addition, complement activation with subsequent tissue damage has been found to be an inflammatory complication of cancer, hemodialysis and the extracorporeal circulation necessary during cardiopulmonary bypass procedures (Wang, Y. et al. (1996) Proc Natl Acad Sci 93:8563-8568; Gasque, P. et al. (1995) J Immunol 155:4882-4889; Rabinovici, R. et al. (1992) J Immunol 149:1744-1750; Belmont, H. et al. (1994) Arthrit Rheum 37:376-383; Elmgreen, J. et al. (1983) Acta Med Scand 214:403-407; Weisman H. et al. (1990) Science 249:146-151; Liu, Z. et al. (1995) J Clin Invest 95:1539-1544; Rinder, C. et al. (1995) J Clin Invest 96:1564-1572).

The discovery of proteins related to human C5a receptor, and the polynucleotides encoding them, satisfies a need in the art by providing new compositions useful in the diagnosis and treatment of disorders associated with inflammation.

#### DISCLOSURE OF THE INVENTION

The present invention features a novel human C5a-like receptor hereinafter designated HCOR and characterized as having similarity to human C5a receptor (GI 115262).

Accordingly, the invention features a substantially purified HCOR having the amino acid sequence shown in SEQ ID NO:1.

One aspect of the invention features isolated and substantially purified polynucleotides that encode HCOR. In a particular aspect, the polynucleotide is the nucleotide sequence of SEQ ID NO:2.

The invention also relates to a polynucleotide sequence comprising the complement of SEQ ID NO:2 or variants thereof. In addition, the invention features polynucleotide sequences

which hybridize under stringent conditions to SEQ ID NO:2.

The invention additionally features nucleic acid sequences encoding polypeptides, oligonucleotides, peptide nucleic acids (PNA), fragments, portions or antisense molecules thereof, and expression vectors and host cells comprising polynucleotides that encode HCOR.

- 5 The invention features a pharmaceutical composition comprising substantially purified HCOR, and the use of this composition for the prevention or treatment of inflammation. The invention also features agonists and antagonists, including antibodies, of HCOR.

### BRIEF DESCRIPTION OF DRAWINGS

Figures 1A, 1B, 1C and 1D show the amino acid sequence (SEQ ID NO:1) and nucleic  
10 acid sequence (SEQ ID NO:2) of HCOR. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co., Ltd., San Bruno, CA).

Figures 2A and 2B shows the amino acid sequence alignments between HCOR (SEQ ID NO:1) and human C5a receptor (GI 115262; SEQ ID NO:3). The alignment was produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

- 15 Figures 3A and 3B show the hydrophobicity plots (MacDNASIS PRO software) for HCOR, SEQ ID NO: 1 and human C5a receptor (GI 115262; SEQ ID NO:3), respectively. The positive X axis reflects amino acid position, and the negative Y axis, hydrophobicity.

### MODES FOR CARRYING OUT THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is  
20 understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a",  
25 "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to the "antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same  
30 meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by

reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

## 5 DEFINITIONS

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand.

Similarly, "amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules.

Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

"Peptide nucleic acid", as used herein, refers to a molecule which comprises an oligomer to which an amino acid residue, such as lysine, and an amino group have been added. These small molecules, also designated anti-gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, P.E. et al. (1993) *Anticancer Drug Des.* 8:53-63).

HCOR, as used herein, refers to the amino acid sequences of substantially purified HCOR obtained from any species, particularly mammalian, including bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

"Consensus", as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, or which has been extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte clone using the GELVIEW™ Fragment Assembly system (GCG, Madison, WI), or which has been both extended and assembled.

A "variant" of HCOR, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of a

glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

5 A "deletion", as used herein, refers to a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the naturally occurring molecule.

10 A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

The term "biologically active", as used herein, refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HCOR,  
15 or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "agonist", as used herein, refers to a molecule which, when bound to HCOR, causes a change in HCOR which modulates the activity of HCOR. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to HCOR.

20 The terms "antagonist" or "inhibitor", as used herein, refer to a molecule which, when bound to HCOR, blocks or modulates the biological or immunological activity of HCOR. Antagonists and inhibitors may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to HCOR.

The term "modulate", as used herein, refers to a change or an alteration in the biological  
25 activity of HCOR. Modulation may be an increase or a decrease in protein activity, a change in binding characteristics, or any other change in the biological, functional or immunological properties of HCOR.

The term "mimetic", as used herein, refers to a molecule, the structure of which is developed from knowledge of the structure of HCOR or portions thereof and, as such, is able to  
30 effect some or all of the actions of C5a receptor-like molecules.

The term "derivative", as used herein, refers to the chemical modification of a nucleic acid encoding HCOR or the encoded HCOR. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative would

encode a polypeptide which retains essential biological characteristics of the natural molecule.

The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

"Amplification" as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY).

10 The term "hybridization", as used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term "hybridization complex", as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (e.g.,  $C_0t$  or  $R_0t$  analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., membranes, filters, chips, pins or glass slides to which cells have been fixed for in situ hybridization).

20 The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, for the sequence "A-G-T" binds to the complementary sequence "T-C-A".

Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands.

The term "homology", as used herein, refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence is one that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid; it is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined



using a hybridization assay (Southern or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence or probe to the target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

As known in the art, numerous equivalent conditions may be employed to comprise either low or high stringency conditions. Factors such as the length and nature (DNA, RNA, base composition) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.), and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.

The term "stringent conditions", as used herein, is the "stringency" which occurs within a range from about  $T_m - 5^\circ\text{C}$  ( $5^\circ\text{C}$  below the melting temperature ( $T_m$ ) of the probe) to about  $20^\circ\text{C}$  to  $25^\circ\text{C}$  below  $T_m$ . As will be understood by those of skill in the art, the stringency of hybridization may be altered in order to identify or detect identical or related polynucleotide sequences.

The term "antisense", as used herein, refers to nucleotide sequences which are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene(s) of interest in a reverse orientation to a viral promoter which permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines with natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. In this manner, mutant phenotypes may be generated. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

The term "portion", as used herein, with regard to a protein (as in "a portion of a given

protein”) refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid. Thus, a protein “comprising at least a portion of the amino acid sequence of SEQ ID NO:1” encompasses the full-length human HCOR and fragments thereof.

5 “Transformation”, as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral  
10 infection, electroporation, lipofection, and particle bombardment. Such “transformed” cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term “antigenic determinant”, as used herein, refers to that portion of a molecule that  
15 makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune  
20 response) for binding to an antibody.

The terms “specific binding” or “specifically binding”, as used herein, in reference to the interaction of an antibody and a protein or peptide, mean that the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the protein; in other words, the antibody is recognizing and binding to a specific protein structure rather than to  
25 proteins in general. For example, if an antibody is specific for epitope “A”, the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled “A” and the antibody will reduce the amount of labeled A bound to the antibody.

The term “sample”, as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acid encoding HCOR or fragments thereof may comprise a cell,  
30 chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern analysis), RNA (in solution or bound to a solid support such as for northern analysis), cDNA (in solution or bound to a solid support), an extract from cells or a tissue, and the like.

The term "correlates with expression of a polynucleotide", as used herein, indicates that the detection of the presence of ribonucleic acid that is similar to SEQ ID NO:2 by northern analysis is indicative of the presence of mRNA encoding HCOR in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.

5 "Alterations" in the polynucleotide of SEQ ID NO: 2, as used herein, comprise any alteration in the sequence of polynucleotides encoding HCOR including deletions, insertions, and point mutations that may be detected using hybridization assays. Included within this definition is the detection of alterations to the genomic DNA sequence which encodes HCOR (e.g., by alterations in the pattern of restriction fragment length polymorphisms capable of hybridizing to  
10 SEQ ID NO:2), the inability of a selected fragment of SEQ ID NO: 2 to hybridize to a sample of genomic DNA (e.g., using allele-specific oligonucleotide probes), and improper or unexpected hybridization, such as hybridization to a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HCOR (e.g., using fluorescent *in situ* hybridization [FISH] to metaphase chromosomes spreads).

15 As used herein, the term "antibody" refers to intact molecules as well as fragments thereof, such as Fa, F(ab')<sub>2</sub>, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind HCOR polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from the transition of RNA or synthesized chemically, and  
20 can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The term "humanized antibody", as used herein, refers to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely  
25 resemble a human antibody, while still retaining the original binding ability.

#### THE INVENTION

The invention is based on the discovery of a novel human C5a-like receptor, (HCOR), the polynucleotides encoding HCOR, and the use of these compositions for the diagnosis, prevention, or treatment of diseases associated with complement activation.

30 Nucleic acids encoding the human HCOR of the present invention were first identified in Incyte Clone 346874 from the thymus tissue cDNA library (THYMNOT02) through a computer-generated search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from extension of the nucleic acid sequences of Incyte Clone 346874

(THYMNOT02).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Figures 1A, 1B 1C and 1D. HCOR is 319 amino acids in length and has chemical and structural homology with human C5a receptor (GI 115626; SEQ ID NO:3). In particular, HCOR and human C5a receptor (GI 115626) share 25% identity. HCOR and human C5a receptor (GI 115626) contain the G-protein-coupled receptor signature motif; this is found in the  $L_{100} - L_{116}$  region of HCOR and in the  $Y_{121} - V_{138}$  region of human C5a receptor (GI 115626). As illustrated by the hydrophobicity plots, Figs. 3A and 3B, HCOR and human C5a receptor both display the seven highly hydrophobic domains that are characteristic of the G-protein-coupled receptor family.

The invention also encompasses HCOR variants. A preferred HCOR variant is one having at least 80%, and more preferably 90%, amino acid sequence identity to the HCOR amino acid sequence (SEQ ID NO:1). A most preferred HCOR variant is one having at least 95% amino acid sequence identity to SEQ ID NO:1.

The invention also encompasses polynucleotides which encode HCOR. Accordingly, any nucleic acid sequence which encodes the amino acid sequence of HCOR can be used to generate recombinant molecules which express HCOR. In a particular embodiment, the invention encompasses the polynucleotide comprising the nucleic acid sequence of SEQ ID NO:2 as shown in Figures 1A, 1B, 1C and 1D.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding HCOR, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring HCOR, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HCOR and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring HCOR under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HCOR or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular

codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HCOR and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

- 5       The invention also encompasses production of DNA sequences, or portions thereof, which encode HCOR and its derivatives, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art at the time of the filing of this application. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding
- 10 HCOR or any portion thereof.

- Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those shown in SEQ ID NO:2, under various conditions of stringency. Hybridization conditions are based on the melting temperature ( $T_m$ ) of the nucleic acid binding complex or probe, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol.
- 15 152:507-511), and may be used at a defined stringency.

- Altered nucleic acid sequences encoding HCOR which are encompassed by the invention include deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent HCOR. The encoded protein may also contain
- 20 deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HCOR. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of HCOR is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively
- 25 charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; phenylalanine and tyrosine.

- Also included within the scope of the present invention are alleles of the genes encoding HCOR. As used herein, an "allele" or "allelic sequence" is an alternative form of the gene which
- 30 may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each

of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such 5 enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 10 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI 377 DNA sequencers (Perkin Elmer).

The nucleic acid sequences encoding HCOR may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be 15 employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round 20 of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186). The primers may be designed using OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, 25 MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR which involves PCR amplification of 30 DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations may also be used to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before performing

PCR.

Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk in genomic DNA (Clontech, Palo Alto, CA). This  
5 process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a  
10 full-length cDNA. Genomic libraries may be useful for extension of sequence into the 5' and 3' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation,  
15 four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. Genotyper™ and Sequence Navigator™, Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially  
20 preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HCOR, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of HCOR in appropriate host cells. Due to the  
25 inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express HCOR.

As will be understood by those of skill in the art, it may be advantageous to produce HCOR-encoding nucleotide sequences possessing non-naturally occurring codons. For example,  
30 codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HCOR encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene  
5 fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HCOR may be ligated to a heterologous sequence to encode a fusion protein.  
10 For example, to screen peptide libraries for inhibitors of HCOR activity, it may be useful to encode a chimeric HCOR protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the HCOR encoding sequence and the heterologous protein sequence, so that HCOR may be cleaved and purified away from the heterologous moiety.

15 In another embodiment, sequences encoding HCOR may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of HCOR, or a portion thereof. For example, peptide synthesis can be performed  
20 using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and  
25 Molecular Principles, WH Freeman and Co., New York, NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, supra). Additionally, the amino acid sequence of HCOR, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

30 In order to express a biologically active HCOR, the nucleotide sequences encoding HCOR or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.



Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HCOR and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in

5 Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY.

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HCOR. These include, but are not limited to, microorganisms such as

10 bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

15 The "control elements" or "regulatory sequences" are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be

20 used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader

25 sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding HCOR, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the

30 use intended for HCOR. For example, when large quantities of HCOR are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional E. coli cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence

encoding HCOR may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of  $\beta$ -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

10 In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding HCOR may be driven by any of a number of promoters. For example, viral promoters such as the 15 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.

An insect system may also be used to express HCOR. For example, in one such system, 25 Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding HCOR may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of HCOR will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant 30 viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which HCOR may be expressed (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In

cases where an adenovirus is used as an expression vector, sequences encoding HCOR may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing HCOR in infected host cells

5 (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HCOR. Such signals include the ATG initiation codon and adjacent  
10 sequences. In cases where sequences encoding HCOR, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading  
15 frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al, (1994) Results Probl. Cell Differ. 20:125-162).

20 In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding  
25 and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express HCOR may be transformed using  
30 expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance

to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These  
5 include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes which can be employed in tk<sup>-</sup> or aprt<sup>-</sup> cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70);  
10 npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S.C. and R.C. Mulligan  
15 (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins,  $\beta$  glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131).

20 Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding HCOR is inserted within a marker gene sequence, recombinant cells containing sequences encoding HCOR can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HCOR under the  
25 control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding HCOR and express HCOR may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and  
30 protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

The presence of polynucleotide sequences encoding HCOR can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or portions or fragments of

polynucleotides encoding HCOR. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding HCOR to detect transformants containing DNA or RNA encoding HCOR. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60  
5 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplimer.

A variety of protocols for detecting and measuring the expression of HCOR, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and  
10 fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HCOR is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. et al. (1983; J. Exp. Med. 158:1211-1216).

15 A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HCOR include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HCOR, or any portions thereof may be cloned  
20 into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH).  
25 Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HCOR may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein  
30 produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HCOR may be designed to contain signal sequences which direct secretion of HCOR through a prokaryotic or eukaryotic cell membrane.

Other recombinant constructions may be used to join sequences encoding HCOR to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the 5 FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and HCOR may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing HCOR and a 10 nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3: 263-281) while the enterokinase cleavage site provides a means for purifying HCOR from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA 15 Cell Biol. 12:441-453).

In addition to recombinant production, fragments of HCOR may be produced by direct peptide synthesis using solid-phase techniques Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide 20 Synthesizer (Perkin Elmer). Various fragments of HCOR may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

### **THERAPEUTICS**

Based on the chemical and structural homology between HCOR (SEQ ID NO:1) and 25 human C5a receptor (SEQ ID NO:3), HCOR appears to play a role in inflammation.

Therefore, in one embodiment, HCOR or a fragment or derivative thereof may be administered to a induce inflammatory response in a subject who has a diminished inflammatory response. A diminished inflammatory response may occur as a result of various conditions including, but not limited to, complement deficiency, immunodeficiency, and impaired wound 30 healing.

In another embodiment, a vector capable of expressing HCOR, or a fragment or a derivative thereof, may also be administered to a subject to induce inflammatory response for any of the conditions listed above.

In another embodiment, antagonists or inhibitors of HCOR may be administered to a subject to prevent inflammation. Types of inflammation may include, but are not limited to, brain demyelination and neurodegeneration; allergic reactions, asthma and adult respiratory distress syndrome, autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, glomerulonephritis; and Crohn's disease; post ischemic myocardial inflammation and necrosis, skin diseases; septic shock, and inflammatory complications of cancer, hemodialysis and extracorporeal circulation, infection and trauma. In one aspect, antibodies which are specific for HCOR may be used directly as an antagonist, or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express HCOR.

10 In another embodiment, a vector expressing antisense of the polynucleotide encoding HCOR may be administered to a subject to treat or prevent inflammation resulting from any of the inflammatory conditions listed in the preceding paragraph.

In other embodiments, any of the therapeutic proteins, antagonists, antibodies, agonists, antisense sequences or vectors described above may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Antagonists or inhibitors of HCOR may be produced using methods which are generally known in the art. In particular, purified HCOR may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HCOR.

The antibodies may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with HCOR or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin,

and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the peptides, fragments, or oligopeptides used to induce antibodies to HCOR have an amino acid sequence consisting of at least five amino acids, and more preferably  
5 at least 10 amino acids. It is also preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HCOR amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

10 Monoclonal antibodies to HCOR may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; Cole,  
15 S.P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature  
20 314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HCOR-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:11120-3).

25 Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

Antibody fragments which contain specific binding sites for HCOR may also be  
30 generated. For example, such fragments include, but are not limited to, the F(ab')<sub>2</sub> fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal



Fab fragments with the desired specificity (Huse, W.D. et al. (1989) Science 254:1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HCOR and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HCOR epitopes is preferred, but a competitive binding assay may also be employed (Maddox, supra).

In another embodiment of the invention, the polynucleotides encoding HCOR, or any fragment thereof, or antisense molecules, may be used for therapeutic purposes. In one aspect, antisense to the polynucleotide encoding HCOR may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HCOR. Thus, antisense molecules may be used to modulate HCOR activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligomers or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding HCOR.

Expression vectors derived from retro viruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors which will express antisense molecules complementary to the polynucleotides of the gene encoding HCOR. These techniques are described both in Sambrook et al. (supra) and in Ausubel et al. (supra).

Genes encoding HCOR can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof which encodes HCOR. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA, or PNA, to the control regions of the gene encoding HCOR, i.e., the promoters, enhancers, and introns. Oligonucleotides derived from the transcription

initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic  
5 advances using triplex DNA have been described in the literature (Gee, J.E. et al. (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage  
10 of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples which may be used include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HCOR.

Specific ribozyme cleavage sites within any potential RNA target are initially identified  
15 by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with  
20 complementary oligonucleotides using ribonuclease protection assays.

Antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription  
25 of DNA sequences encoding HCOR. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible  
30 modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such

as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally  
5 suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods which are well known in the art.

Any of the therapeutic methods described above may be applied to any subject in need of  
10 such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of  
15 HCOR, antibodies to HCOR, mimetics, agonists, antagonists, or inhibitors of HCOR. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs  
20 or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

25 In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton,  
30 PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets,

pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be

permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

5       The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine. 0.1%-2% sucrose, and  
10 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HCOR, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions  
15 wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or  
20 pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HCOR or fragments thereof, antibodies of HCOR, agonists, antagonists or inhibitors of HCOR,  
25 which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which  
30 exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage

form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

#### DIAGNOSTICS

In another embodiment, antibodies which specifically bind HCOR may be used for the diagnosis of conditions or diseases characterized by expression of HCOR, or in assays to monitor patients being treated with HCOR, agonists, antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for HCOR include methods which utilize the antibody and a label to detect HCOR in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring HCOR are known in the art and provide a basis for diagnosing altered or abnormal levels of HCOR expression. Normal or standard values for HCOR expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to HCOR under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, but preferably by photometric means. Quantities of HCOR expressed in subject, control and disease, samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the

parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HCOR may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs. The polynucleotides may be used to  
5 detect and quantitate gene expression in biopsied tissues in which expression of HCOR may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of HCOR, and to monitor regulation of HCOR levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting  
10 polynucleotide sequences, including genomic sequences, encoding HCOR or closely related molecules, may be used to identify nucleic acid sequences which encode HCOR. The specificity of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will  
15 determine whether the probe identifies only naturally occurring sequences encoding HCOR, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the HCOR encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and derived from the  
20 nucleotide sequence of SEQ ID NO:2 or from genomic sequence including promoter, enhancer elements, and introns of the naturally occurring HCOR.

Means for producing specific hybridization probes for DNAs encoding HCOR include the cloning of nucleic acid sequences encoding HCOR or HCOR derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and  
25 may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as <sup>32</sup>P or <sup>35</sup>S, or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

30 Polynucleotide sequences encoding HCOR may be used for the diagnosis of conditions or diseases which are associated with expression of HCOR. Examples of such conditions or diseases include brain demyelination and neurodegeneration; allergic reactions, asthma and adult respiratory distress syndrome, autoimmune disorders such as rheumatoid arthritis, systemic lupus

erythematosis, glomerulonephritis; and Crohn's disease; post ischemic myocardial inflammation and necrosis; skin diseases; septic shock,, and inflammatory complications of cancer, hemodialysis and extracorporal circulation; and inflammatory responses necessary to kill microorganisms, remove damaged tissues, and prepare the region for tissue repair or  
5 regeneration. The polynucleotide sequences encoding HCOR may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilizing fluids or tissues from patient biopsies to detect altered HCOR expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HCOR may be useful in assays  
10 that detect activation or induction of various cancers, particularly those mentioned above. The nucleotide sequences encoding HCOR may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the biopsied or extracted sample  
15 is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding HCOR in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an  
20 individual patient.

In order to provide a basis for the diagnosis of disease associated with expression of HCOR, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes HCOR, under conditions suitable for  
25 hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

30 Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to



months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HCOR may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically, or produced from a recombinant source. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'→3') and another with antisense (3'←5'), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of HCOR include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby, P.C. et al. (1993) J. Immunol. Methods, 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 229-236). The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In another embodiment of the invention, the nucleic acid sequences which encode HCOR may also be used to generate hybridization probes which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. Such techniques include FISH, FACS, or artificial chromosome constructions, such as yeast artificial chromosomes, bacterial artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C.M. (1993) Blood Rev. 7:127-134, and Trask, B.J. (1991) Trends Genet. 7:149-154.

FISH (as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in the

1994 Genome Issue of Science (265:1981f). Correlation between the location of the gene encoding HCOR on a physical chromosomal map and a specific disease, or predisposition to a specific disease, may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences  
5 between normal, carrier, or affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human  
10 chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. et al. (1988) Nature 336:577-580), any sequences mapping  
15 to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

In another embodiment of the invention, HCOR, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening libraries of compounds in any of a variety of  
20 drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between HCOR and the agent being tested, may be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in  
25 published PCT application WO84/03564. In this method, as applied to HCOR large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with HCOR, or fragments thereof, and washed. Bound HCOR is then detected by methods well known in the art. Purified HCOR can also be coated directly onto plates for use in the aforementioned drug screening techniques.  
30 Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HCOR specifically compete with a test compound for

binding HCOR. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HCOR.

In additional embodiments, the nucleotide sequences which encode HCOR may be used in any molecular biology techniques that have yet to be developed, provided the new techniques  
5 rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

### INDUSTRIAL APPLICABILITY

#### 10 I THYMNOT02 cDNA Library Construction

The THYMNOT02 cDNA library was constructed from thymus tissue (lot #93-122) obtained from the Keystone Skin Bank, International Institute for the Advancement of Medicine (Exton PA). The frozen tissue was ground in a mortar and pestle and lysed immediately in a buffer containing guanidinium isothiocyanate. The lysate was extracted twice with phenol  
15 chloroform at pH 8.0 and centrifuged over a CsCl cushion using an Beckman SW28 rotor in a Beckman L8-70M Ultracentrifuge (Beckman Instruments). The RNA was precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in water and DNase treated for 15 min at 37°C. The RNA was isolated using the Qiagen Oligotex kit (QIAGEN Inc, Chatsworth CA) and used to construct the cDNA library.

20 First strand cDNA synthesis was accomplished using an oligo d(T) primer/linker which also contained an XhoI restriction site. Second strand synthesis was performed using a combination of DNA polymerase I, *E. coli* ligase and RNase H, followed by the addition of an EcoRI adaptor to the blunt ended cDNA. The EcoRI adapted, double-stranded cDNA was then digested with XhoI restriction enzyme and fractionated on Sephacryl S400 to obtain sequences  
25 which exceeded 1000 bp in size. The size selected cDNAs were inserted into the LambdaZap® vector system (Stratagene); and the vector, which contains the pBluescript™ phagemid (Stratagene), was transformed into cells of *E. coli*, strain XL1-BlueMRF™ (Stratagene).

The phagemid forms of individual cDNA clones were obtained by the in vivo excision process. Enzymes from both pBluescript and a cotransformed f1 helper phage nicked the DNA,  
30 initiated new DNA synthesis, and created the smaller, single-stranded circular phagemid DNA molecules which contained the cDNA insert. The phagemid DNA was released, purified, and used to reinfect fresh host cells (SOLR, Stratagene).

## II Isolation and Sequencing of cDNA Clones

Plasmid DNA was purified using the Miniprep Kit (Catalogue # 77468, Advanced Genetic Technologies Corporation, Gaithersburg MD). The recommended protocol included with the kit was employed except for the following changes. Each of the 96 wells was filled with  
5 only 1 ml of sterile Terrific Broth (Catalog # 22711, LIFE TECHNOLOGIES™, Gaithersburg, MD) with carbenicillin at 25 mg/L and glycerol at 0.4%. After the wells were inoculated, the bacteria were cultured for 24 hours and lysed with 60  $\mu$ l of lysis buffer. A centrifugation step (Beckman GS-6R @2900 rpm for 5 min; Beckman Instruments) was performed before the contents of the block were added to the primary filter plate. The optional step of adding  
10 isopropanol to TRIS buffer was not routinely performed. After the last step in the protocol, samples were transferred to a Beckman 96-well block for storage.

The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems  
15 377 or 373 DNA Sequencing Systems (Perkin Elmer) and reading frame was determined.

## III Homology Searching of cDNA Clones and Their Deduced Proteins

Each cDNA was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 sequence analysis system. In this algorithm, Pattern Specification Language (TRW Inc, Los Angeles, CA) was used to  
20 determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to  
25 distinguish regions of homology from chance matches. Smith-Waterman alignments were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT- 670 sequence analysis system using the methods similar to those used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for  
30 sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

BLAST, which stands for Basic Local Alignment Search Tool (Altschul, S.F. (1993) J.

Mol. Evol. 36:290-300; Altschul et al. (1990) J. Mol. Biol. 215:403-410), was used to search for local sequence alignments. BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. BLAST is  
5 useful for matches which do not contain gaps. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence  
10 and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence  
15 whose match satisfies E is reported in the program output.

#### IV Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound (Sambrook et al., supra).

20 Analogous computer techniques using BLAST (Altschul, S.F. 1993 and 1990, supra) are used to search for identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

25 The basis of the search is the product score which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact  
30 within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript

encoding HCOR occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

## 5 V Extension of HCOR-Encoding Polynucleotides to Full Length or to Recover Regulatory Sequences

Full length HCOR-encoding nucleic acid sequence (SEQ ID NO:2) is used to design oligonucleotide primers for extending a partial nucleotide sequence to full length or for obtaining 5' or 3', intron or other control sequences from genomic libraries. One primer is synthesized to  
 10 initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence in the sense direction (XLF). Primers are used to facilitate the extension of the known sequence "outward" generating amplicons containing new, unknown nucleotide sequence for the region of interest. The initial primers are designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be 22-30 nucleotides in length, to have a GC  
 15 content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations is avoided.

The original, selected cDNA libraries, or a human genomic library are used to extend the sequence; the latter is most useful to obtain 5' upstream regions. If more extension is necessary  
 20 or desired, additional sets of primers are designed to further extend the known region.

By following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA) and the  
 25 following parameters:

	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
	Step 3	68° C for 6 min
	Step 4	94° C for 15 sec
30	Step 5	65° C for 1 min
	Step 6	68° C for 7 min
	Step 7	Repeat step 4-6 for 15 additional cycles
	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
35	Step 10	68° C for 7:15 min
	Step 11	Repeat step 8-10 for 12 cycles

Step 12            72° C for 8 min  
Step 13            4° C (and holding)

A 5-10  $\mu$ l aliquot of the reaction mixture is analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in  
5 extending the sequence. Bands thought to contain the largest products are selected and removed from the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc., Chatsworth, CA). After recovery of the DNA, Klenow enzyme is used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

10            After ethanol precipitation, the products are redissolved in 13  $\mu$ l of ligation buffer, 1  $\mu$ l T4-DNA ligase (15 units) and 1  $\mu$ l T4 polynucleotide kinase are added, and the mixture is incubated at room temperature for 2-3 hours or overnight at 16° C. Competent *E. coli* cells (in 40  $\mu$ l of appropriate media) are transformed with 3  $\mu$ l of ligation mixture and cultured in 80  $\mu$ l of SOC medium (Sambrook et al., supra). After incubation for one hour at 37° C, the whole  
15 transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook et al., supra) containing 2x Carb. The following day, several colonies are randomly picked from each plate and cultured in 150  $\mu$ l of liquid LB/2x Carb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5  $\mu$ l of each overnight culture is transferred into a non-sterile 96-well plate and after dilution 1:10 with water,  
20 5  $\mu$ l of each sample is transferred into a PCR array.

For PCR amplification, 18  $\mu$ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the following conditions:

25	Step 1	94° C for 60 sec
	Step 2	94° C for 20 sec
	Step 3	55° C for 30 sec
	Step 4	72° C for 90 sec
	Step 5	Repeat steps 2-4 for an additional 29 cycles
30	Step 6	72° C for 180 sec
	Step 7	4° C (and holding)

Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid, and sequenced.

## VI Labeling and Use of Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250  $\mu$ Ci of [ $\gamma$ - $^{32}$ P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont NEN<sup>®</sup>, Boston, MA). The labeled oligonucleotides are substantially purified with Sephadex G-25 superfine resin column (Pharmacia & Upjohn). A portion containing 10<sup>7</sup> counts per minute of each of the sense and antisense oligonucleotides is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II; DuPont NEN<sup>®</sup>).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR<sup>™</sup> film (Kodak, Rochester, NY) is exposed to the blots in a Phosphorimager cassette (Molecular Dynamics, Sunnyvale, CA) for several hours, hybridization patterns are compared visually.

## VII Antisense Molecules

Antisense molecules to the HCOR-encoding sequence, or any part thereof, is used to inhibit in vivo or in vitro expression of naturally occurring HCOR. Although use of antisense oligonucleotides, comprising about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. An oligonucleotide based on the coding sequences of HCOR, as shown in Figures 1A, 1B, 1C and 1D, is used to inhibit expression of naturally occurring HCOR. The complementary oligonucleotide is designed from the most unique 5' sequence as shown in Figures 1A, 1B, 1C and 1D and used either to inhibit transcription by preventing promoter binding to the upstream nontranslated sequence or translation of an HCOR-encoding transcript by preventing the ribosome from binding. Using an appropriate portion of the signal and 5' sequence of SEQ ID NO:2, an effective antisense oligonucleotide includes any 15-20 nucleotides spanning the region which translates into the signal or 5' coding sequence of the polypeptide as shown in Figures 1A, 1B, 1C and 1D.



### VIII Expression of HCOR

Expression of HCOR is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector, pSport, previously used for the generation of the cDNA library is used to express HCOR in *E. coli*. Upstream of the cloning site, this vector contains a promoter for  $\beta$ -galactosidase, followed by sequence containing the amino-terminal Met, and the subsequent seven residues of  $\beta$ -galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with IPTG using standard methods produces a fusion protein which consists of the first eight residues of  $\beta$ -galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of HCOR into the bacterial growth media which can be used directly in the following assay for activity.

### IX Demonstration of HCOR Activity

HCOR activity is assessed by ligand-stimulated activation of phosphatidyl inositol-specific phospholipase C (PLC). COS cells (ATCC) are co-transfected with HCOR expression plasmids and plasmids encoding the G protein  $\alpha_{16}$  subunit. Following activation by the C5a ligand (Sigma), HCOR signaling via G protein  $\alpha_{16}$  stimulation of the endogenous COS cell PLC is measured and compared to normalized control values. For this assay, the transfected cells are incubated for 24hrs with inositol-free DMEM containing 10uCi/ml [ $^3$ H]inositol and 10% fetal bovine serum. This medium is then replaced with inositol-free DMEM, 10mM LiCl, 0-150nM C5a and the cells are incubated for 30 minutes at 37°C. The reactions are terminated by addition of an equal volume of 10% HClO<sub>4</sub> containing 4mg/ml phytic acid and incubated for 30 minutes at 0 or -20°C. The inositol phosphates are purified by chromatography on Dowex-1 (Bio-Rad), quantified by liquid scintillation counting and compared with control values (Gerard, N. (1995) J Biol Chem 270:18077-18082).

### X Production of HCOR Specific Antibodies

HCOR that is substantially purified using PAGE electrophoresis (Sambrook, supra), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols. The amino acid sequence deduced from SEQ ID NO:2 is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Selection of appropriate epitopes, such as those near the C-terminus or in

hydrophilic regions, is described by Ausubel et al. (supra), and others.

Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-  
5 hydroxysuccinimide ester (MBS; Ausubel et al., supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.

#### **XI Purification of Naturally Occurring HCOR Using Specific Antibodies**

10 Naturally occurring or recombinant HCOR is substantially purified by immunoaffinity chromatography using antibodies specific for HCOR. An immunoaffinity column is constructed by covalently coupling HCOR antibody to an activated chromatographic resin, such as CnBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

15 Media containing HCOR is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HCOR (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HCOR binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HCOR is collected.

#### **20 XII Identification of Molecules Which Interact with HCOR**

HCOR or biologically active fragments thereof are labeled with <sup>125</sup>I Bolton-Hunter reagent (Bolton et al. (1973) Biochem. J. 133: 529). Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HCOR, washed and any wells with labeled HCOR complex are assayed. Data obtained using different concentrations of HCOR are  
25 used to calculate values for the number, affinity, and association of HCOR with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit  
30 of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are

intended to be within the scope of the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- (i) APPLICANT: INCYTE PHARMACEUTICALS, INC.
- (ii) TITLE OF THE INVENTION: NOVEL HUMAN C5A-LIKE RECEPTOR
- (iii) NUMBER OF SEQUENCES: 3
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
  - (B) STREET: 3174 Porter Drive
  - (C) CITY: Palo Alto
  - (D) STATE: CA
  - (E) COUNTRY: USA
  - (F) ZIP: 94304
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Diskette
  - (B) COMPUTER: IBM Compatible
  - (C) OPERATING SYSTEM: DOS
  - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
  - (A) PCT APPLICATION NUMBER: To Be Assigned
  - (B) FILING DATE: Herewith
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/791,974
  - (B) FILING DATE: 31-JAN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Billings, Lucy J.
  - (B) REGISTRATION NUMBER: 36,749
  - (C) REFERENCE/DOCKET NUMBER: PF-0198 pct
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 650-855-0555
  - (B) TELEFAX: 650-845-4166
  - (C) TELEX:

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 319 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (vii) IMMEDIATE SOURCE:
  - (A) LIBRARY: Consensus
  - (B) CLONE: 346874
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Thr Asn Ser Ser Phe Phe Cys Pro Val Tyr Lys Asp Leu Glu Pro  
 1 5 10 15

```

Phe Thr Tyr Phe Phe Tyr Leu Val Phe Leu Val Gly Ile Ile Gly Ser
                20                25                30
Cys Phe Ala Thr Trp Ala Phe Ile Gln Lys Asn Thr Asn His Arg Cys
                35                40                45
Val Ser Ile Tyr Leu Ile Asn Leu Leu Thr Ala Asp Phe Leu Leu Thr
                50                55                60
Leu Ala Leu Pro Val Lys Ile Val Val Asp Leu Gly Val Ala Pro Trp
        65                70                75                80
Lys Leu Lys Ile Phe His Cys Gln Val Thr Ala Cys Leu Ile Tyr Ile
                85                90                95
Asn Met Tyr Leu Ser Ile Ile Phe Leu Ala Phe Val Ser Ile Asp Arg
                100                105                110
Cys Leu Gln Leu Thr His Ser Cys Lys Ile Tyr Arg Ile Gln Glu Pro
                115                120                125
Gly Phe Ala Lys Met Ile Ser Thr Val Val Trp Leu Met Val Leu Leu
                130                135                140
Ile Met Val Pro Asn Met Met Ile Pro Ile Lys Asp Ile Lys Glu Lys
        145                150                155                160
Ser Asn Val Gly Cys Met Glu Phe Lys Lys Glu Phe Gly Arg Asn Trp
                165                170                175
His Leu Leu Thr Asn Phe Ile Cys Val Ala Ile Phe Leu Asn Phe Ser
                180                185                190
Ala Ile Ile Leu Ile Ser Asn Cys Leu Val Ile Arg Gln Leu Tyr Arg
                195                200                205
Asn Lys Asp Asn Glu Asn Tyr Pro Asn Val Lys Lys Ala Leu Ile Asn
                210                215                220
Ile Leu Leu Val Thr Thr Gly Tyr Ile Ile Cys Phe Val Pro Tyr His
        225                230                235                240
Ile Val Arg Ile Pro Tyr Thr Leu Ser Gln Thr Glu Val Ile Thr Asp
                245                250                255
Cys Ser Thr Arg Ile Ser Leu Phe Lys Ala Lys Glu Ala Thr Leu Leu
                260                265                270
Leu Ala Val Ser Asn Leu Cys Phe Asp Pro Ile Leu Tyr Tyr His Leu
                275                280                285
Ser Lys Ala Phe Arg Ser Lys Val Thr Glu Thr Phe Ala Ser Pro Lys
        290                295                300
Glu Thr Lys Ala Gln Lys Glu Lys Leu Arg Cys Glu Asn Asn Ala
        305                310                315

```

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1257 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Consensus
- (B) CLONE: 346874

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

CAGCTCATGC TTCTCTGAAG ACTTGCAGCA AGGCTTGCTG AGGCTCACAG AAGATAGCCC      60
CAGTGTTTTG GAGTGGTTTT GAATGTGATT CTGAGATCAG ACTGACTGAG CTGGAATCCT      120
GGCTTTATAT CTTACCAGCT ACWCAACCTT GGAGTCTTAG AAATTTTTC TTTTCARTAA      180
GCAGTCATCC TTACTTTCCC TCAAGATGAC AAACAGTTCG TTCTTCTGCC CAGTTTATAA      240
AGATCTGGAG CCATTCACGT ATTTTTTTTA TTTAGTTTTC CTTGTTGGAA TTATTGGAAG      300
TTGTTTTGCA ACCTGGGCTT TTATACAGAA GAATACGAAT CACAGGTGTG TGAGCATCTA      360
CTTAATTAAT TTGCTTACAG CCGATTTCCT GCTTACTCTG GCATTACCAG TGAAAATTGT      420
TGTTGACTTG GGTGTGGCAC CTTGGAARCT GAAGATATTC CACTGCCAAG TAACAGCCTG      480

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CCTCATCTAT ATCAATATGT ATTTATCAAT TATCTTCTTA GCATTTGTCA GCATTGACCG 540
CTGTCTTCAG CTGACACACA GCTGCAAGAT CTACCGAATA CAAGAACCCG GGTTTGCCAA 600
AATGATATCA ACCGTTGTGT GGCTAATGGT CCTTCTTATA ATGGTGCCAA ATATGATGAT 660
TCCCATCAAA GACATCAAGG AAAAGTCAAA TGTGGGTTGT ATGGAGTTTA AAAAGGAATT 720
TGGAAGAAAT TGGCATTTCG TGACAAATTT CATATGTGTA GCAATATTTT TAAATTTCTC 780
AGCCATCATT TTAATATCCA ATTGCCTTGT AATTCGACAG CTCTACAGAA ACAAAGATAA 840
TGAAAATTAC CCAAATGTGA AAAAGGCTCT CATCAACATA CTTTGTAGTA CCACGGGCTA 900
CATCATATGC TTTGTTTCCTT ACCACATTGT CCGAATCCCG TATACCCTCA GCCAGACAGA 960
AGTCATAACT GATTGCTCAA CCAGGATTTT ACTCTTCAAA GCCAAAGAGG CTACACTGCT 1020
CCTGGCTGTG TCGAACCTGT GCTTTGATCC TATCCTGTAC TATCACCTCT CAAAAGCATT 1080
CCGCTCAAAG GTCACGTAGA CTTTTCCTC ACCTAAAGAG ACCAAGGCTC AGAAAGAAAA 1140
ATTAAGATGT GAAAATAATG CATAAAAGAC AGGATTTTTT GTGCTACCAA TTCTGGCCTT 1200
ACTGGACCAT AAAGTTAATT ATAGCTTTGA AAGATAAAAA AAAAAAAAAA AAAAAAA 1257

```

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 350 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank
- (B) CLONE: 115262

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Asn Ser Phe Asn Tyr Thr Thr Pro Asp Tyr Gly His Tyr Asp Asp
 1           5           10           15
Lys Asp Thr Leu Asp Leu Asn Thr Pro Val Asp Lys Thr Ser Asn Thr
 20           25           30
Leu Arg Val Pro Asp Ile Leu Ala Leu Val Ile Phe Ala Val Val Phe
 35           40           45
Leu Val Gly Val Leu Gly Asn Ala Leu Val Val Trp Val Thr Ala Phe
 50           55           60
Glu Ala Lys Arg Thr Ile Asn Ala Ile Trp Phe Leu Asn Leu Ala Val
 65           70           75           80
Ala Asp Phe Leu Ser Cys Leu Ala Leu Pro Ile Leu Phe Thr Ser Ile
 85           90           95
Val Gln His His Trp Pro Phe Gly Gly Ala Ala Cys Ser Ile Leu
100          105          110
Pro Ser Leu Ile Leu Leu Asn Met Tyr Ala Ser Ile Leu Leu Leu Ala
115          120          125
Thr Ile Ser Ala Asp Arg Phe Leu Leu Val Phe Lys Pro Ile Trp Cys
130          135          140
Gln Asn Phe Arg Gly Ala Gly Leu Ala Trp Ile Ala Cys Ala Val Ala
145          150          155          160
Trp Gly Leu Ala Leu Leu Thr Ile Pro Ser Phe Leu Tyr Arg Val
165          170          175
Val Arg Glu Glu Tyr Phe Pro Pro Lys Val Leu Cys Gly Val Asp Tyr
180          185          190
Ser His Asp Lys Arg Arg Glu Arg Ala Val Ala Ile Val Arg Leu Val
195          200          205
Leu Gly Phe Leu Trp Pro Leu Leu Thr Leu Thr Ile Cys Tyr Thr Phe
210          215          220
Ile Leu Leu Arg Thr Trp Ser Arg Arg Ala Thr Arg Ser Thr Lys Thr
225          230          235          240
Leu Lys Val Val Val Ala Val Val Ala Ser Phe Phe Ile Phe Trp Leu
245          250          255

```

Pro	Tyr	Gln	Val	Thr	Gly	Ile	Met	Met	Ser	Phe	Leu	Glu	Pro	Ser	Ser
			260					265					270		
Pro	Thr	Phe	Leu	Leu	Leu	Asn	Lys	Leu	Asp	Ser	Leu	Cys	Val	Ser	Phe
		275					280					285			
Ala	Tyr	Ile	Asn	Cys	Cys	Ile	Asn	Pro	Ile	Ile	Tyr	Val	Val	Ala	Gly
		290					295				300				
Gln	Gly	Phe	Gln	Gly	Arg	Leu	Arg	Lys	Ser	Leu	Pro	Ser	Leu	Leu	Arg
305					310					315					320
Asn	Val	Leu	Thr	Glu	Glu	Ser	Val	Val	Arg	Glu	Ser	Lys	Ser	Phe	Thr
				325					330					335	
Arg	Ser	Thr	Val	Asp	Thr	Met	Ala	Gln	Lys	Thr	Gln	Ala	Val		
			340					345					350		

What is claimed is:

1. A substantially purified novel human C5a-like protein (HCOR) comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof.
2. An isolated and purified polynucleotide sequence encoding the human C5a-like  
5 protein of claim 1.
3. A polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of claim 2.
4. A hybridization probe comprising the polynucleotide sequence of claim 2.
5. An isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or  
10 variants thereof.
6. A polynucleotide sequence which is complementary to the polynucleotide sequence of claim 2 or variants thereof.
7. A hybridization probe comprising the polynucleotide sequence of claim 6.
8. An expression vector containing the polynucleotide sequence of claim 2.
- 15 9. A host cell containing the vector of claim 8.
10. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 the method comprising the steps of:
  - a) culturing the host cell of claim 9 under conditions suitable for the expression of the polypeptide; and
  - 20 b) recovering the polypeptide from the host cell culture.
11. A pharmaceutical composition comprising a substantially purified human C5a-like protein having the amino acid sequence of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.
12. A purified antibody which binds specifically to the polypeptide of claim 1.
- 25 13. A purified agonist which specifically binds to and modulates the activity of the polypeptide of claim 1.
14. A purified antagonist which specifically binds to and modulates the activity of the polypeptide of claim 1.
15. A method for treating inflammation comprising administering to a subject in need  
30 of such treatment an effective amount of the antagonist of claim 14.
16. A method for detection of a polynucleotide encoding human C5a-like protein in a biological sample comprising the steps of:
  - a) hybridizing the polynucleotide of claim 6 to nucleic acid material of a



biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a polynucleotide encoding human C5a-like protein in said biological sample.

- 5        17. An isolated amino acid sequence having at least 95% sequence identity to SEQ ID NO:1.

1/7

9	18	27	36	45	54
AGC TCA TGC TTC TCT TCT GAA GAC TTG CAG CAA GGC TTG CTG AGG CTC ACA GAA GAT					
63	72	81	90	99	108
AGC CCC AGT GTT TTG GAG TGG TTT TGA ATG TGA TTC TGA GAT CAG ACT GAC TGA					
117	126	135	144	153	162
GCT GGA ATC CTG GCT TTA TAT TAT CTT ACC AGC TAC WCA ACC TTG GAG TCT TAG AAA					
171	180	189	198	207	216
TTT TTT CTT TTC ART AAG CAG TCA TCC TTA CTT TCC CTC AAG ATG ACA AAC AGT					
225	234	243	252	261	270
TCG TTC TTC TGC CCA GTT TAT AAA GAT CTG GAG CCA TTC ACG TAT TTT TTT TAT					
S F C P V Y K D L E P F T Y F Y					
279	288	297	306	315	324
TTA GTT TTC CTT GTT GGA ATT ATT GGA AGT TGT TTT GCA ACC TGG GCT TTT ATA					
L V F L V G I I G S C F A T W A F I					
333	342	351	360	369	378
CAG AAG AAT ACG AAT CAC AGG TGT GTG AGC ATC TAC TTA ATT AAT TTG CTT ACA					
Q K N T N H R C V S I Y L I N L L T					

FIGURE 1A

387	GCC GAT TTC	396	CTG CTT ACT	405	GCA TTA CCA	414	GTG AAA ATT	423	GTT GTT GAC	432	TTG GGT
A D F	L L L	T T L	A A L	P P V	K K I	V V V	D D L	G G			
441	GTC GCA CCT	450	TGG AAR CTG	459	ATA AAG ATA	468	TGC CAA GTA	477	GCC ACA	486	CTC ATC
V A P	W K L	K L I	F H C	Q V T	A C L	I					
495	TAT ATC AAT	504	TAT TTA TCA	513	ATC ATT ATC	522	GCA TTT	531	AGC AGC	540	GAC CGC
Y I N	M Y L	S I I	F L A	F V S	I D R						
549	TGT CTT CAG	558	CTG ACA CAC	567	TGC AAG ATC	576	TAC CGA	585	GAA CCC	594	GGG TTT
C L Q	L T H	S C K	I Y R	I Q F	G						
603	GCC AAA ATG	612	TCA ACC GTT	621	GTG TGG CTA	630	ATG GTC	639	ATA ATA	648	GTG CCA
A K M	I S T	V V W	L M V	L L I	M V P						
657	AAT ATG ATG	666	ATC CCC ATC	675	GAC AAA GAC	684	AAG TCA	693	GTG GGT	702	TGT ATG
N M M	I I P	K D I	K E S	N V G	C M						
711	GAG TTT AAA	720	TTT GAA AGA	729	AAT TGG CAT	738	TTG CTG	747	AAT TTC	756	ATA TGT
E F K	K E F	G R N	H W L	L T F	I C						

FIGURE 1B

765	774	783	792	801	810
GTA GCA ATA TTT TTA AAT TTC TCA GCC ATC ATT TTA ATA TCC AAT TGC CTT GTA					
V A I F L N F S A I I L I S N C L V					
819	828	837	846	855	864
ATT CGA CAG CTC TAC AGA AAC AAA GAT AAT GAA AAT TAC CCA AAT GTG AAA AAG					
I R Q L Y R N K D N E N Y P N V K K					
873	882	891	900	909	918
GCT CTC ATC AAC ATA CTT TTA GTG ACC ACG GGC TAC ATC ATA TGC TTT GTT CCT					
A L I N I L L V T T G Y I I C F V P					
927	936	945	954	963	972
TAC CAC ATT GTC CGA ATC CCG TAT ACC CTC AGC CAG ACA GAA GTC ATA ACT GAT					
Y H I V R I P Y T L S Q T E V I T D					
981	990	999	1008	1017	1026
TGC TCA ACC AGG ATT TCA CTC TTC AAA GCC AAA GAG GCT ACA CTG CTC CTG GCT					
C S T R I S L F K A A E A T L L A					
1035	1044	1053	1062	1071	1080
GTG TCG AAC CTG TGC TTT GAT CCT ATC CTC TAC TAT CAC CTC TCA AAA GCA TTC					
V S N L C F D P I L L Y Y H L S K A F					
1089	1098	1107	1116	1125	1134
CGC TCA AAG GTC ACT GAG ACT TTT GCC TCA CCT AAA GAG ACC AAG GCT CAG AAA					
R S K V T E T F A S P K E T K A Q K					

FIGURE 1C

1143	1152	1161	1170	1179	1188
GAA AAA TTA AGA TGT GAA AAT AAT GCA TAA AAG ACA GGA TTT TTT GTG CTA CCA					
E K L R C E N N A					
1197	1206	1215	1224	1233	1242
ATT CTG GCC TTA CTG GAC CAT AAA GTT AAT TAT AGC TTT GAA AGA TAA AAA AAA					
1251					
AAA AAA AAA AAA AA					

FIGURE 1D

1	M	T	N	S	S	F	F	C	P	V	Y	-	-	-	K	D	L	E	P	F	T	Y	-	-	-	-	-	-	-	-	346874
1	M	N	S	F	N	Y	T	T	P	D	Y	G	H	Y	D	D	K	D	T	L	D	L	N	T	P	V	D	K	T	S	GI 115262
20	-	-	-	-	-	-	-	-	-	-	-	F	F	Y	L	V	F	L	V	G	I	I	G	S	C	F	A	T	W	346874	
31	N	T	L	R	V	P	D	I	L	A	L	V	I	F	A	V	V	F	L	V	G	V	L	G	N	A	L	V	V	W	GI 115262
38	-	-	A	F	I	Q	K	N	T	N	H	R	C	V	S	I	Y	L	I	N	L	L	T	A	D	F	L	L	T	L	346874
61	V	T	A	F	E	A	K	R	T	I	N	-	-	-	A	I	W	F	L	N	L	A	V	A	D	F	L	S	C	L	GI 115262
66	A	L	P	V	K	I	V	V	D	L	G	V	A	P	W	K	L	K	I	F	H	C	Q	V	T	A	C	L	I	Y	346874
88	A	L	P	I	L	F	T	S	I	V	Q	H	H	W	P	F	G	G	A	A	C	S	I	L	P	S	L	I	L	GI 115262	
96	I	N	M	Y	L	S	I	I	F	L	A	F	V	S	I	D	R	C	L	Q	L	T	H	S	-	-	C	K	I	Y	346874
118	L	N	M	Y	A	S	I	L	L	A	T	I	S	A	D	R	F	L	L	V	F	K	P	I	W	C	Q	N	F	GI 115262	
124	R	I	Q	E	P	G	F	A	K	M	I	S	T	V	V	W	L	M	V	L	L	I	M	V	P	N	M	M	I	P	346874
148	R	-	-	G	A	G	L	A	W	I	A	C	A	V	A	W	G	L	A	L	L	T	I	P	S	F	L	Y	R	GI 115262	
154	I	K	D	I	K	E	K	-	-	-	S	N	V	G	C	-	M	E	F	K	K	E	F	G	R	N	W	H	L	L	346874
176	V	-	-	V	R	E	Y	F	P	P	K	V	L	C	G	V	D	Y	S	H	D	K	R	R	E	R	A	V	A	GI 115262	

FIGURE 2A

180 T N F I C V A I F L N F - S A I I L I S N C L V I R Q L Y R 346874  
 204 I - - - V R L V L G F L W P L L T L T I C Y T F I - L L R GI 115262  
  
 209 N K D N E N Y P N V K K A L I N I L L V T T G Y I I C F V P 346874  
 229 T W S R R A - T R S T K T L K V V V A V V A S F F I F W L P GI 115262  
  
 239 Y H I V R I P Y T L S Q T E V I T D C S T R I S L F K A K E 346874  
 258 Y Q V T G I M M S F - - - L E P S S P T F L L L N K L D S GI 115262  
  
 269 A T L L L A V S N L C F D P I L Y Y H L S K A F R S K V T E 346874  
 284 L C V S F A Y I N C C I N P I I Y V V A G Q G F Q G R L R K GI 115262  
  
 299 T F A S - - - - - P K E T K A - - - - Q K E K 346874  
 314 S L P S L L R N V L T E E S V V R E S K S F T R S T V D T M GI 115262  
  
 313 L R C E N N A 346874  
 344 A Q K T Q A V GI 115262

FIGURE 2B

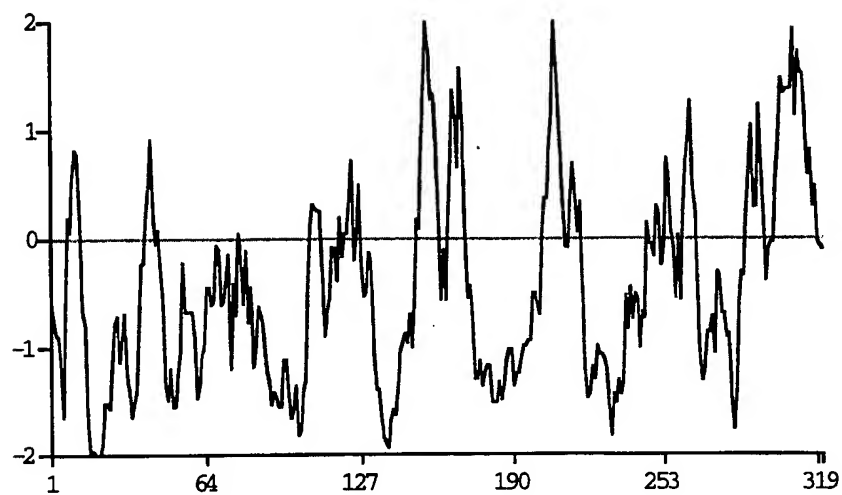


FIGURE 3A

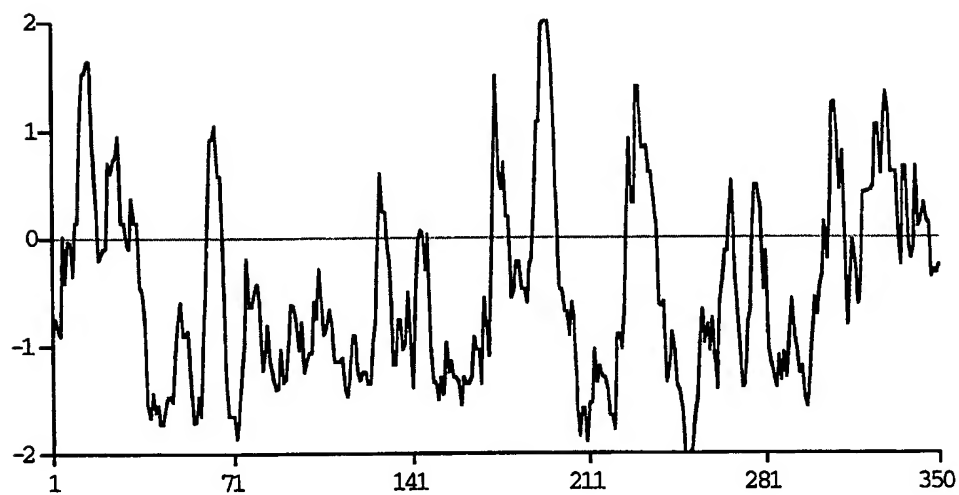


FIGURE 3B



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01182

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C12Q1/68 C12N5/10 A61K38/17  
C07K16/28 C07K14/72

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A01K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GERARD C ET AL: "C5A ANAPHYLATOXIN AND ITS SEVEN TRANSMEMBRANE-SEGMENT RECEPTOR" ANNUAL REVIEW OF IMMUNOLOGY, vol. 12, 1 January 1994, pages 775-808, XP000579211 cited in the application see the whole document ---	1
A	EMBL Mouse EST Project, Accession Number AA162789, Created 20 DEC-1996, mr29d12.r1 Soares mouse 3NbMS Mus musculus cDNA clone 598871 5' XP002065266 see the whole document --- -/--	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*A\* document member of the same patent family

Date of the actual completion of the international search

18 May 1998

Date of mailing of the international search report

03.07.98

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/01182

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 05226 A (HUMAN GENOME SCIENCES INC ;LI YI (US); ROSEN CRAIG A (US)) 22 February 1996	1
P,X	--- JACOBS, K.A. ET AL.: "A genetic selection for isolating cDNAs encoding secreted proteins" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES., vol. 198, 1 October 1997, BARKING GB, pages 284-296, XP002065265 see the whole document	1-9,16, 17
E	--- WO 98 07859 A (GENETICS INST) 26 February 1998 see the whole document	1
E	--- EP 0 837 128 A (SMITHKLINE BEECHAM CORP) 22 April 1998 see the whole document -----	1

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 98/01182

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claim 15 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Application No

PCT/US 98/01182

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9605226 A	22-02-1996	AU 7566594 A EP 0787149 A	07-03-1996 06-08-1997
WO 9807859 A	26-02-1998	US 5723315 A	03-03-1998
EP 0837128 A	22-04-1998	NONE	